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Enterobacter hormaechei, a New Species of the Family *Enterobacteriaceae* Formerly Known as Enteric Group 75

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The name *Enterobacter hormaechei* is proposed for a new species of the family *Enterobacteriaceae*, formerly called Enteric Group 75, which consists of 23 strains, 22 of which were isolated from humans. DNAs from 12 *E. hormaechei* strains tested were highly related to the type strain (ATCC 49162) by DNA hybridization, using the hydroxyapatite method (80 to 97% in 60°C reactions; 80 to 90% in 75°C reactions). The strains were most closely related (50 to 63%) to *Enterobacter cloacae*, *Enterobacter dissolvens*, *Enterobacter taylorae*, and *Enterobacter nimipressuralis*. *E. hormaechei* strains were positive within 48 h for the following: Voges-Proskauer test; citrate utilization (Simmons and Christensen); urea hydrolysis (87%); ornithine decarboxylase; growth in potassium cyanide (KCN); malonate utilization; production of acid from D-glucose, L-arabinose, cellobiose, dulcitol (87%), D-galactose, maltose, D-mannitol, D-mannose, L-rhamnose, sucrose, trehalose, and D-xylose; acid production from mucate; nitrate reduction; and *o*-nitrophenyl-β-D-galactopyranoside. Delayed positive reactions were seen in tests for arginine dihydrolase, gas from D-glucose, acid from α-methyl-D-glucoside, and acetate utilization. *E. hormaechei* was negative in tests for indole production; H_2S production; phenylalanine deaminase; lysine decarboxylase; gelatin hydrolysis; acid production from D-adonitol, D-arabitol, erythritol, glycerol, *i*(*myo*)-inositol, melibiose, raffinose, and D-sorbitol; esculin hydrolysis; DNase; lipase; and tyrosine clearing. Variable reactions occurred in tests for methyl red, motility, and tartrate. All strains tested were susceptible or moderately susceptible to amikacin, azlocillin, cefotaxime, ceftazidime, ceftriaxone, chloramphenicol, gentamicin, mezlocillin, moxalactam, piperacillin, trimethoprim-sulfamethoxazole, sulfisoxazole, thienamycin, tobramycin, and trimethoprim. All strains tested were resistant to nitrofurantoin; the majority were resistant to ampicillin, cefoxitin, and cephalothin. Four isolates were from blood; most other isolates were from wounds or sputum.

During the study in which *Enterobacter taylorae* was defined (5), three additional hybridization groups were noted (J. J. Farmer, G. R. Fanning, C. M. O'Hara, C. F. Riddle, F. W. Hickman-Brenner, and D. J. Brenner, unpublished data). These were arbitrarily designated Enteric Groups 74, 75, and 76. The largest of these, Enteric Group 75, contained 11 strains that were sent in to the Enteric Bacteriology Laboratories, Centers for Disease Control, for identification between 1973 and 1984. Twelve additional strains were received from 1985 through 1987, three of which were blood isolates. Because of the increase in isolates of Enteric Group 75 and of blood isolates, we studied this group further, concluding that it represents a new species, for which the name *Enterobacter hormaechei* is proposed.

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MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1. All were maintained in semisolid tryptic soy agar deeps at room temperature.

Media and biochemical tests. The biochemical tests were performed on conventional media by the methods of Edwards and Ewing (2), with some modifications by Hickman and Farmer et al. (3, 6). Incubations were at 35°C and test results were read at 24 h, 48 h, and 7 days, unless otherwise noted. Commercial media were used whenever possible.

Antibiotic susceptibility. Antibiotic susceptibilities were determined for 13 strains by the Kirby-Bauer disk diffusion method (9). MICs were determined by using a broth microdilution method and cation-supplemented Mueller-Hinton broth (10).

DNA methods. The preparation, isolation, and purification of labeled and unlabeled DNA, the method used for DNA reassociation, and the method used to separate single-stranded and double-stranded DNA on hydroxyapatite have been described elsewhere (1, 5). The guanine-plus-cytosine content (G+C) of DNA was determined for two strains spectrophotometrically by thermal denaturation (8).

TABLE 1. Strains of *E. hormaechei*

CDC strain no.	Location of sender	Source of specimen	Sex, age (yr), clinical history of patient
1735-73	Connecticut	Frog liver and gut	
3883-73	Washington	Stool	Female, 7
0992-77^T (ATCC 49162)	California	Sputum	Male
0332-78	Wisconsin	Exudate, face	
3020-78	Missouri	Wound, foot	
2573-81	Tennessee	Urine	Male, 66, indwelling Foley catheter, nondraining
4012-83	California	Sputum	Male, 85, pneumonia, diabetes
4104-83	Louisiana	Sputum	Male, 89, acute bronchitis
4227-83	California	Wound, leg	Female, 88, femoral bypass, urinary tract infection
4795-84	Tennessee	Gallbladder	Female, gallbladder surgery
4911-84	Missouri	Blood	Female, newborn
4010-85	Missouri	Exudate, abdomen	Male, 15, Crohn's disease
4237-85	Florida	Sputum	Female, 29, pneumonia
4521-86 (ATCC 49163)	Hawaii	Blood	Male, 75, bacteremia, metastatic lung cancer
4589-86	Georgia	Blood	Female, 61
4004-87	Texas	Wound, abdomen	
4022-87	Michigan	Exudate, ear	
4024-87	Maine	Peritoneal fluid	Male, 70
4030-87	Michigan	Abdominal fluid	
4039-87	Michigan	Sputum	
4041-87	Indiana	Burn	
4058-87	Florida	Blood	
4069-87	Canada	Unknown	

RESULTS AND DISCUSSION

E. hormaechei sp. nov. (hor.maé.che.i) N.L. gen. nov. *hormaechei* is named in honor of Estenio Hormaeche, a Uruguayan microbiologist who (with P. R. Edwards) proposed and defined the genus *Enterobacter* (7). The *E. hormaechei* strains that were studied and pertinent clinical information are shown in Table 1. The facts that four strains were isolated from blood and that several others were isolated from hospital patients suggest that *E. hormaechei* may have clinical significance. The type strain of *E. hormaechei* is 0992-77 (ATCC 49162). It was isolated from the sputum of a man in California in 1977.

DNA hybridization. Labeled DNA from *E. hormaechei* 0992-77^T was 80 to 92% related (average, 87%) to unlabeled DNA from the 12 other *E. hormaechei* strains tested in 60°C reactions (Table 2). Divergence within the related sequences was 0.5 to 3.5%, and relatedness in 75°C reactions was 80 to 96% (average, 86%). *E. hormaechei* was most closely related to *Enterobacter cloacae* (63%), *Enterobacter dissolvens*

TABLE 2. DNA relatedness of *E. hormaechei* strains

Source of unlabeled DNA	Relatedness (%) to labeled DNA from *E. hormaechei* 0992-77^T		
	At 60°C	*D*[a]	At 75°C
Enterobacter hormaechei			
0992-77^T	100	0.0	100
4911-84	92	0.5	96
3020-78	91	1.5	89
4237-85	91	2.0	85
1735-73	90	1.0	85
4795-84	88	0.5	88
0332-78	88	1.5	89
2573-81	87	1.5	81
4012-83	87	3.0	80
3883-73	86	3.5	81
4010-85	86	2.0	91
4104-83	83	1.0	85
4227-83	80	1.5	84
Enterobacter cloacae 1347-71	63	9.0	50
Enterobacter dissolvens ATCC 23373^T	63	9.0	42
Enterobacter taylorae 2126-81^T	57	10.0	35
Enterobacter nimipressuralis EN-1^T	52	11.0	29
Enterobacter agglomerans 5378-71	50		30
Other *Enterobacter* species (16 strains)	14–46		
Erwinia herbicola 9571-82	25		
Erwinia rhapontici ER106	23		
Erwinia stewartii 9573-82	20		
Erwinia uredovora 9574-82	20		
Erwinia amylovora EA178	20		
Erwinia mallotivora 2851	17		
Erwinia quercina EQ102	17		
Erwinia salicis ES102	16		
Erwinia ananas 9570-82	15		
Erwinia tracheiphila ET106	8		
Erwinia carotovora 495	6		
Other members of the family *Enterobacteriaceae* (52 strains)	4–34		

Continued

TABLE 2—*Continued*

[a] Divergence within related sequences, calculated on the assumption that each 1°C decrease in the thermal stability of a DNA duplex is caused by 1% of unpaired bases within that duplex. *D* was calculated to the nearest 0.5%.

TABLE 3. Biochemical reactions of 23 strains of *E. hormaechei*

Test[a]	Cumulative % positive at:			Reaction of type strain ATCC 49162[b]
	24 h	48 h	7 days	
Indole production		0		−
Methyl red		57		+
Voges-Proskauer		100		+
Citrate, Simmons	78	96	96	+
Hydrogen sulfide (on TSI Agar)	0	0	0	−
Urea, Christensen	70	87	87	+
Phenylalanine deaminase	4			−
Lysine, Moeller	0	0	0	−
Arginine, Moeller	70	78	83	+
Ornithine, Moeller	91	91	96	+
Motility	48	52	52	+
Gelatin hydrolysis (22°C)	0	0	17	−
Growth in KCN	96	100	100	+
Malonate utilization	96	100	100	+
D-Glucose				
Acid production	100	100	100	+
Gas production	78	83	87	+
Acid production from:				
D-Adonitol	0	0	0	−
L-Arabinose	100	100	100	+
D-Arabitol	0	0	0	−
Cellobiose	100	100	100	+
Dulcitol	78	87	87	+
Erythritol	0	0	0	−
D-Galactose	100	100	100	+
Glycerol	4	4	70	+[5]
i(*myo*)-Inositol	0	0	0	−
Lactose	0	9	96	+[5]
Maltose	100	100	100	+
D-Mannitol	100	100	100	+
D-Mannose	100	100	100	+
Melibiose	0	0	0	−
α-Methyl-D-glucoside	70	83	91	+[2]
Raffinose	0	0	0	−
L-Rhamnose	100	100	100	+
Salicin	0	44	96	+[3]
D-Sorbitol	0	0	0	−
Sucrose	100	100	100	+
Trehalose	100	100	100	+
D-Xylose	96	96	96	+
Mucate, acid production	87	96	100	+
Tartrate, Jordan	13	13	13	−
Esculin hydrolysis	0	0	57	+[7]
Acetate utilization	30	74	87	+[2]
Hydrogen sulfide (PIA)[c]	0	0	0	−
Citrate, Christensen	96	100	100	+
$NO_3^- \rightarrow NO_2^-$	100			+
Oxidase	0			−
DNase (25°C)	0	0	0	−
Lipase (corn oil)	0	0	0	−
ONPG[d]	95	95	100	+
Yellow pigment (25°C)	0	0	0	−
Tyrosine clearing	0	0	0	−

[a] Incubation at 35°C unless otherwise specified.
[b] Symbols: −, negative; +, positive. The superscript numbers indicate the days the reactions became positive.
[c] Peptone iron agar.
[d] *o*-Nitrophenyl-β-D-galactopyranoside.

(63%), *E. taylorae* (57%), *Enterobacter nimipressuralis* (52%), and *Enterobacter agglomerans* hybridization group XI (50%). Relatedness to other *Enterobacter* species was <46% and to other members of the family *Enterobacteriaceae* was <34% (Table 2). *E. hormaechei* DNA (two strains) contains 58.5 ± 0.3 mol% G+C.

Description of *E. hormaechei* sp. nov. Strains of *E. hormaechei* are gram-negative, oxidase-negative, fermentative, nonpigmented rods with the general characteristics of the family *Enterobacteriaceae* and of the genus *Enterobacter* (Table 3). Biochemically, *E. hormaechei* was closest to *E. taylorae*. Tests for use in differentiating *E. hormaechei* from all other *Enterobacter* species are given in Table 4.

E. hormaechei strains were tested against 25 antimicrobial agents (Table 5).

TABLE 4. Differentiation of *Enterobacter* species

Species	% Reactions positive[a] in test											
	Lysine, Moeller	Urea	Motility	Methyl red	Lactose	Sucrose	D-Sorbitol	Raffinose	Melibiose	α-Methyl-D-glucoside	Dulcitol	Esculin
E. hormaechei	0	87	52	5	9	100	0	0	0	83	87	0
E. aerogenes	98	2	97	5	95	100	100	99	99	95	5	98
E. agglomerans	0	20	85	50	40	75	30	30	50	7	15	60
E. amnigenus, biogroup 1	0	0	92	7	70	100	9	100	100	55	0	91
E. amnigenus, biogroup 2	0	0	100	65	35	0	100	0	100	100	0	100
E. asburiae	0	85	1	100	75	100	100	69	9	94	0	94
E. cloacae	0	65	95	5	93	97	95	97	90	85	15	30
E. dissolvens	0	100	0	0	0	100	100	100	100	100	0	100
E. gergoviae	90	95	90	5	55	98	0	97	97	2	0	97
E. intermedium	0	0	89	100	100	65	100	100	100	100	100	100
E. nimipressuralis	0	0	0	100	0	0	100	0	100	100	0	100
E. sakazakii	0	1	90	5	99	100	0	99	100	96	5	100
E. taylorae	0	1	99	5	10	0	1	0	0	1	0	90

[a] Percentages for all species except *E. hormaechei*, *E. dissolvens*, and *E. nimipressuralis* are from charts by Farmer et al. (4); *E. dissolvens* and *E. nimipressuralis* percentages are those of the respective type strains. Results were obtained as positive reactions on conventional media incubated at 35°C for 48 h.

TABLE 5. Antimicrobial susceptibility of *E. hormaechei* by broth microdilution

Antimicrobial agent	No. of strains with MIC[a] (μg/ml) of:						
	≤1	2	4	8	16	32	>32
Gentamicin	23						
Tobramycin	22	1					
Amikacin	12	8	3				
Cephalothin		1		2			20
Cefoxitin			3				20
Cefamandole	4	5	3	2		1	8
Cefotaxime	19				3		1
Moxalactam	19	1	2	1			
Cefoperazone	10	9			2		2
Ceftazidime	18	1			1	2	1
Ceftriaxone	18	1		1	2	1	
Ampicillin	1	1	2	1	4	3	11
Carbenicillin			1	13	3	1	5
Ticarcillin	2	9	4	1	2	1	4
Piperacillin	5	11	2	4			1
Mezlocillin	1	7	10	2	1	1	1
Azlocillin			4	4	8	2	5
Thienamycin	23						
Tetracycline	1	1	15	5			1
Chloramphenicol			2	15	6		
Sulfamethoxazole-trimethoprim	22			1			
Sulfisoxazole			1	6	4	6	6
Trimethoprim	18	2	1	1	1		
Nalidixic acid		2	15	4	1	1	
Nitrofurantoin			1			1	21[b]

[a] The line indicates the MIC breakpoint for resistant isolates.
[b] All with MICs of >64 μg/ml.

LITERATURE CITED

1. **Brenner, D. J., A. C. McWhorter, J. K. L. Knutson, and A. G. Steigerwalt.** 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. J. Clin. Microbiol. **15**:1133–1140.
2. **Edwards, P. R., and W. H. Ewing.** 1972. Identification of *Enterobacteriaceae*, 3rd ed. Burgess Publishing Co., Minneapolis.
3. **Farmer, J. J., III, M. A. Asbury, F. W. Hickman, D. J. Brenner, and the *Enterobacteriaceae* Study Group.** 1980. *Enterobacter sakazakii*: a new species of "*Enterobacteriaceae*" isolated from clinical specimens. Int. J. Syst. Bacteriol. **30**:569–584.
4. **Farmer, J. J., III, B. R. Davis, F. W. Hickman-Brenner, A. McWhorter, G. P. Huntley-Carter, M. A. Asbury, C. Riddle, H. G. Wathen-Grady, C. Elias, G. R. Fanning, A. G. Steigerwalt, C. M. O'Hara, G. K. Morris, P. B. Smith, and D. J. Brenner.** 1985. Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. J. Clin. Microbiol. **21**:46–76.
5. **Farmer, J. J., III, G. R. Fanning, B. R. Davis, C. M. O'Hara, C. Riddle, F. W. Hickman-Brenner, M. A. Asbury, V. A. Lowery III, and D. J. Brenner.** 1985. *Escherichia fergusonii* and *Enterobacter taylorae*, two new species of *Enterobacteriaceae* isolated from clinical specimens. J. Clin. Microbiol. **21**:77–81.
6. **Hickman, F. W., and J. J. Farmer III.** 1978. *Salmonella typhi*: identification, antibiograms, serology, and bacteriophage typing. Am. J. Med. Technol. **44**:1149–1159.
7. **Hormaeche, E., and P. R. Edwards.** 1960. A proposed genus *Enterobacter*. Int. Bull. Bacteriol. Nomencl. Taxon. **10**:71–74.
8. **Marmur, J., and P. Doty.** 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5**:109–118.
9. **National Committee for Clinical Laboratory Standards.** 1983. Performance standards for antimicrobic disc susceptibility tests. Approved standard M2-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
10. **National Committee for Clinical Laboratory Standards.** 1983. Standard methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Tentative standard M7-T. National Committee for Clinical Laboratory Standards, Villanova, Pa.